CHEMICAL CONTROL OF MICROORGANISMS, Evaluation of Antiseptics and Disinfectants

Background and introduction:

At home, at work, or at a hospital, we try and to “eliminate” microbes in our environment. Is this possible, probably not but we use a variety of chemicals such as antimicrobial soaps, disinfectants, antiseptic solutions to decontaminate a wide variety of surfaces that include your skin and tissues. Some of theses examples include items that you can buy at your local market such as Lysol and Clorox. You can also visit your local pharmacist and buy first aid treatments like isopropanol, hydrogen peroxide and Listerine. Whereas your hospitals will buy other cleaners like as Zephiran and Cepacol.

These chemical agents will work in one of two ways. They can alter the cell wall or the plasma membrane of the cell or the can interfere with proteins and nucleic acids inside the cell. They will either have a “static” or “cidal” effect on the cell.

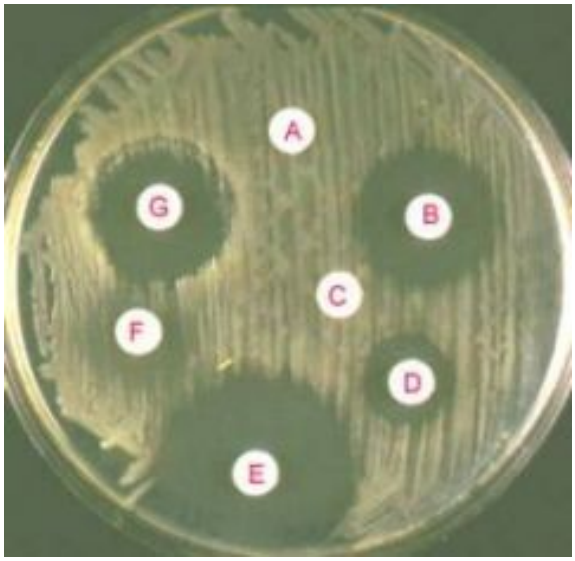
Pre-lab questions:

1. What does the term “static” mean?
2. What does the term “cidal” mean?
3. What is the plasma membrane composed of?
4. Is there a difference between “sanitization” and “sterilization”?

In this experiment you will try and identify which chemical agent works best on which microbe based on the disk diffusion assay. With this assay, you will take small paper disks and soak them in a given chemical. They will then be placed on a plate that is already inoculated with a specific microbe to form a bacterial lawn. The agar plates are allowed to incubate with the bacteria and chemicals. This will allow for the bacterial lawn to grow and will be enough time for the given chemicals to diffuse in the agar. As you should already suspect, as the chemical diffuses into the agar, it will become less concentrated.

What you will be observing after the plates are incubated is a clear **zone of inhibition** around the paper disk if the growth of the microbes has been inhibited. You will then measure the diameter of this zone of inhibition and can then determine if the microbe is sensitive to the specific chemical or not. Below is a picture of

bacterial lawn with paper discs that have been soak in chemicals will look like. Please observe the clear zones around the papers. That is the zone on inhibition that you are looking for.



5. Why does it matter which chemical agent is applied? Shouldn't they all work the same on all microbes?
6. What is a bacterial lawn?

We will be testing four or more different chemicals today using the disk diffusion assay. You can bring a specific chemical to lab to test for its effectiveness on these microbes.

MATERIALS NEEDED:

- Wax Pencils (6 per Table)

- Disinfectant Bottles (2 per table)

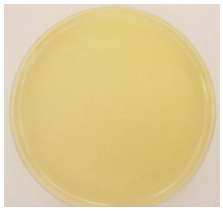
- Test Tube Racks (2 per Table)

- Sterile Cotton Swabs (30 per Table)

- Bunsen Burners and Hoses (2 of each per table)

- “Waste” 500ml Beakers (1 per table)

- Nutrient Broth Cultures of *S. aureus* (1 Per Table)
- Nutrient Broth Cultures of *E. coli* (1 per Table)
- Nutrient Broth Cultures of *P. aeruginosa* (1 per Table)
- 3 Mueller Hinton Agar dishes.



- Forceps (3 per Table)

- Isopropanol bottles (1 per Table)

- Rulers (3 per Table) (Day 2)

- Sterile Filter Paper (30 per table)

- The following antiseptics and disinfectants are needed for each bench
    - 5% iodine
    - 409 spray
    - H_2O_2
    - Hand-sanitizer
    - Hand soap
    - Isopropanol
    - Lab disinfectant
    - Laundry detergent
    - Scope

CULTURES NEEDED:

Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa

PROCEDURE:

1. With a marker, divide the bottom of the plate into sections that will be used for each chemical that you will use.

2. Label the Mueller-Hinton agar plate with you name, date, class, and microorganism that you intend on using on it.

3. Select a broth culture of *E. coli*. Please resuspend any microbes that may settled on the bottom of the tube. Take a sterile swab and dip it into the broth tube that has *E. coli* and completely inoculate the surface of the agar.

4. Rotate the plate 90° and repeat step 3. The entire surface of the agar needs to be swabbed. There should be a bacterial lawn growing after one day incubation.

5. Using sterile forceps, dip a sterile paper disk in your first chemical solution.

6. Make sure to allow the excess solution to drip off. Place the disk in the center of first section. ***GENTLY*** depress each disk onto the agar. Do not puncture the agar.

7. Repeat procedure 5 and 6 with each chemical solution that you plan on using.

8. Repeat steps 2-7 for *S. aureus* and *P. aeruginosa*.

9. Invert you plates (agar side up) and incubate them at 35 degrees Celsius for 48 hours.

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5. Tape the plate, and incubate at 37 °C for 48 hours.

RESULTS:

Using a plastic ruler, measure the diameter in millimeters (mm) of the zone of inhibition for each chemical tested. Record your results in the chart below.

Chemical	***E. coli* Zone of Inhibition (mm)**	***S. aureus* Zone of Inhibition (mm)**	***P. aeruginosa* Zone of Inhibition (mm)**
Hand Soap			
Hydrogen peroxide			
Clorox cleaner			
Lab disinfectant			
Isopropanol			

Post-lab questions:

1. Describe the mechanisms of action of Soap, isopropanol, hydrogen peroxide, and Clorox bleach against bacteria.

2. Based on your results, which chemical is most effective against *E. coli*? Which is least effective against *E. coli*? Why and why not?

3. Which is most effective against *S. aureus*? Which is least effective against *S. aureus*? Why and why not?

4. Which is most effective against *P. aeruginosa*? Which is least effective against *P. aeruginosa*? Why and why not?

5. Which chemical would you choose if you wanted maximum effectiveness against the microbes *E. coli* and *S. aureus*?